

(FILE 'USPAT' ENTERED AT 08:33:53 ON 05 MAY 1998)

L1 29 S (ISOPENTENYL DIPHOSPHATE OR DIMETHYLALLYL DIPHOSPHATE OR  
GE  
L2 85 S FARNESYL DIPHOSPHATE  
L3 85 S L1 OR L2  
L4 0 S L3 AND PRENYL DIPHOSPHATE SYNTHASE#  
L5 2 S L3 AND DIPHOSPHATE SYNTHASE#  
L6 2 S DIPHOSPHATE SYNTHASE#  
L7 2 S L5 OR L6

FILE 'JPO' ENTERED AT 08:36:57 ON 05 MAY 1998

L8 2 S L7

FILE 'EPOABS' ENTERED AT 08:37:15 ON 05 MAY 1998

L9 3 S L7

1. US 05443978A, Aug. 22, 1995, Chrysanthemyl **\*\*diphosphate\*\***  
**\*\*synthase\*\***, corresponding genes and use in pyrethrin synthesis; SUZANNE  
R ELLENBERGER, et al., C12N 9/10; C12N 15/54

US 05443978A

L9: 1 of 3

DATE FILED: Jun. 25, 1993

ABSTRACT:

This invention provides a purified chrysanthemyl **\*\*diphosphate\*\***  
**\*\*synthase\*\*** (CDS), a method for the purification of CDS from  
Chrysanthemum cinerariaefolium, and an amino acid sequence of the  
isolated CDS. Also provided is a cDNA encoding the CDS, a nucleotide  
sequence of the CDS gene, and a derived amino acid sequence of the  
encoded CDS protein. The CDS gene is useful in the enzymatic production  
of the natural stereospecific configuration of chrysanthemyl derivatives  
which are useful for the synthesis of pyrethrins, pyrethroids,  
derivatives thereof, as well as other classes of metabolites.

2. EP 00674000A2, Sep. 27, 1995, Geranylgeranyl **\*\*diphosphate\*\***  
**\*\*synthase\*\*** and DNA coding therefor.; TOKUZO C O TOYOTA JIDO NISHINO, et  
al., C12N 9/10; C12N 15/54

EP 00674000A2

L9: 2 of 3

DATE FILED: Mar. 23, 1995

ABSTRACT:

&emsp;&emsp;&emsp;&emsp;&DNA coding for thermostable geranylgeranyl  
diphosphate (GGDP) synthase derived from Sulfolobus acidocaldarius is  
provided. The DNA is useful for production of GGDP synthase, which is, in  
turn, useful for production of GGDP. &lt;IMAGE&gt;

3. WO 09500634A1, Jan. 5, 1995, CHRYSANTHEMYL \*\*DIPHOSPHATE\*\*  
\*\*SYNTHASE\*\*, CORRESPONDING GENES AND USE IN PYRETHRIN SYNTHESIS; SUZANNE  
R ELLENBERGER, et al., C12N 9/10; C12N 15/54; C12N 15/03; C12N 15/04;  
C12N 15/05; C12N 15/06; C12N 15/07; C12N 15/28

WO 09500634A1

L9: 3 of 3

DATE FILED: Jun. 21, 1994

ABSTRACT:

This invention provides a purified chrysanthemyl \*\*diphosphate\*\*  
\*\*synthase\*\* (CDS), a method for the purification of CDS from  
Chrysanthemum cinerariaefolium, and a partial amino acid sequence of the  
isolated CDS. Also provided is a cDNA encoding the CDS, a nucleotide  
sequence of the CDS gene, and a derived amino acid sequence of the  
encoded CDS protein. The CDS gene is useful in the enzymatic production  
of the natural stereospecific configuration of chrysanthemyl derivatives  
which are useful for the synthesis of pyrethrins, pyrethroids,  
derivatives thereof, as well as other classes of metabolites.

1. JP409065878A , Mar. 11, 1997, LONG-CHAIN PRENYL \*\*DIPHOSPHATE\*\*  
\*\*SYNTHASE\*\*, ONUMA, SHINICHI, et al.,  
INT-CL: C12N9/10; C07H21/04; C12N1/19; C12N15/09; C12P9/00

JP409065878A

L8: 1 of 2

DATE FILED: Sep. 1, 1995

ABSTRACT:

PROBLEM TO BE SOLVED: To obtain a new prenyl \*\*diphosphate\*\*  
\*\*synthase\*\* comprising an amino acid at a specific part in a  
geranylgeranyl \*\*diphosphate\*\* \*\*synthase\*\* derived from Sulfolobus  
acidocaldarius, capable of a prenyl \*\*diphosphate\*\* \*\*synthase\*\* such as  
a steroid precursor, etc.

SOLUTION: This new variation type enzyme comprises at least one of Phe  
at the 77 position, Met at the 85 position, Val at the 99 position, Tyr  
at the 101 position, Phe at the 118 position, Arg at the 199 position and  
Asp at the 312 position replaced with another amino acid in a  
geranylgeranyl \*\*diphosphate\*\* \*\*synthase\*\* derived from Sulfolobus  
acidocaldarius and is capable of forming a  $\geq 25$ C prenyl \*\*diphosphate\*\*  
\*\*synthase\*\*. Otherwise a new variation type enzyme is modified by  
replacement with, deficiency in and/or addition of one or a few amino  
acids, maintains the enzyme activity. The enzyme is useful for  
synthesizing a long-chain prenyl \*\*diphosphate\*\* \*\*synthase\*\* such as a  
steroid precursor, carotenoid, etc.

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2. JP407308193A , Nov. 28, 1995, GERANYLGERANYL \*\*DIPHOSPHATE\*\*  
\*\*SYNTHASE\*\* AND DNA ENCODING THE SAME; OOTO, TOKU, et al.,  
INT-CL: C12N15/09; C12N1/21; C12P7/04  
ADDITIONAL-INT-CL: C12N9/10

JP407308193A

L8: 2 of 2

DATE FILED: Nov. 25, 1994

ABSTRACT:

PURPOSE: To obtain the subject new DNA encoding geranylgeranyl diphosphate (GGDP)synthase derived from Sulfolobus-acidocaldarius (ATCC 3,3909), capable of producing GGDP useful as a biosynthetic intermediate for carotenoid, diterpene, rubber, etc.

CONSTITUTION: This new DNA has an amino acid sequence of the formula, encodes geranylgeranyl diphosphate (GGDP)synthase derived from Sulfolobus- acidocaldarius (ATCC 33,909) and is capable of producing GGDP useful as a biosynthetic intermediate for carotenoid, diterpene, rubber, etc. The DNA is obtained by collecting a chromosome DNA from Sulfolobus-acidocaldarius, screening a gene library prepared by a conventional procedure and treating a plasmid recovered from a positive clone of the screening with a restriction enzyme.

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FILE 'USPAT' ENTERED AT 08:38:11 ON 05 MAY 1998

1. 5,622,779, Apr. 22, 1997, Cellular factors that regulate the expression of genes encoding proteins involved in cholesterol homeostasis and methods of using same; Roger Davis, 424/520 [IMAGE AVAILABLE]

US PAT NO: 5,622,779 [IMAGE AVAILABLE] L7: 1 of 2  
DATE FILED: May 27, 1994

ABSTRACT:

The present invention provides a substantially purified cellular factor that can regulate the expression of genes that encode proteins involved in cholesterol metabolism. The invention also provides methods of obtaining the cellular factors of the invention in a substantially purified form. The invention further provides a method of using a cellular factor of the invention to reduce cholesterol levels in a subject having hypercholesterolemia comprising administering a cellular factor to the subject.

2. 5,443,978, Aug. 22, 1995, Chrysanthemyl \*\*diphosphate\*\* \*\*synthase\*\*, corresponding genes and use in pyrethrin synthesis; Suzanne R. Ellenberger, et al., 435/193, 252.3, 252.33, 320.1; 536/23.2, 23.6 [IMAGE AVAILABLE]

US PAT NO: 5,443,978 [IMAGE AVAILABLE]  
DATE FILED: Jun. 25, 1993

L7: 2 of 2

**ABSTRACT:**

This invention provides a purified chrysanthemyl **\*\*diphosphate\*\***  
**\*\*synthase\*\*** (CDS), a method for the purification of CDS from  
Chrysanthemum cinerariaefolium, and an amino acid sequence of the  
isolated CDS. Also provided is a cDNA encoding the CDS, a nucleotide  
sequence of the CDS gene, and a derived amino acid sequence of the  
encoded CDS protein. The CDS gene is useful in the enzymatic production  
of the natural stereospecific configuration of chrysanthemyl derivatives  
which are useful for the synthesis of pyrethrins, pyrethroids,  
derivatives thereof, as well as other classes of metabolites.

STN

(FILE 'HOME' ENTERED AT 08:49:38 ON 05 MAY 1998)

FILE 'CAPLUS, MEDLINE, BIOSIS, EMBASE' ENTERED AT 08:49:51 ON 05  
MAY 1998

L1 1210 S (FARNESYL DIPHOSPHATE OR ISOPENTENYL DIPHOSPHATE OR DIM  
L2 389 S L1 AND DIPHOSPHATE SYNTHASE#  
L3 2 S L1 AND PRENYL DIPHOSPHATE SYNTHASE#  
L4 108 S L2 AND (MUTANT# OR MUTATION# OR VARIANT#)  
L5 26 S L2 AND ((MUTANT# OR MUTATION# OR VARIANT#)(5A)(DIPHOSP  
L6 26 S L5 OR L3  
L7 15 DUPLICATE REMOVE L6 (11 DUPLICATES REMOVED)

=> d 1-15 ibib ab

L7 ANSWER 1 OF 15 CAPLUS COPYRIGHT 1998 ACS

ACCESSION NUMBER: 1998:94627 CAPLUS

DOCUMENT NUMBER: 128:151099

TITLE: \*\*\*Mutants\*\*\* of a \*\*\*prenyl\*\*\*  
\*\*\*diphosphate\*\*\* \*\*\*synthase\*\*\* of  
Sulfolobus acidocaldarius for preparation of  
short-chain prenyl diphosphate

INVENTOR(S): Nakane, Hiroyuki; Oto, Akira; Onuma, Shinichi;  
Hirooka, Kazutake; Nishino, Tokuzou

PATENT ASSIGNEE(S): Toyota Motor Corp., Japan

SOURCE: Jpn. Kokai Tokkyo Koho, 16 pp.

CODEN: JKXXAF

NUMBER DATE

PATENT INFORMATION: JP 10033184 A2 980210 Heisei

APPLICATION INFORMATION: JP 96-213211 960724

DOCUMENT TYPE: Patent

LANGUAGE: Japanese

AB Disclosed are \*\*\*mutants\*\*\* of a \*\*\*prenyl\*\*\*  
\*\*\*diphosphate\*\*\* \*\*\*synthase\*\*\* of Sulfolobus acidocaldarius  
prepd. by substitution and/or insertion in the Asp-rich domain in  
region II (Markush structure given). The reaction products of the  
\*\*\*prenyl\*\*\* \*\*\*diphosphate\*\*\* \*\*\*synthase\*\*\* are  
\*\*\*farnesyl\*\*\* \*\*\*diphosphate\*\*\*. Demonstrated was the prepn.  
of 5 geranyl- \*\*\*geranyl\*\*\* \*\*\*diphosphate\*\*\*  
\*\*\*synthase\*\*\* \*\*\*mutants\*\*\* of S. acidocaldarius by  
site-specific mutation, which were (1) 78-Thr.fwdarw.Phe and  
81-His.fwdarw.Ala; (2) 78-Thr.fwdarw.Phe and 81-His.fwdarw.Leu; (3)  
77-Phe.fwdarw.Tyr, 78-Thr.fwdarw.Phe, and 81-His.fwdarw.Leu; (4)  
77-Phe.fwdarw.Tyr, 78-Thr.fwdarw.Phe, and 81-His.fwdarw.Ala; or (5)  
77-Phe.fwdarw.Tyr, 78-Thr.fwdarw.Ser, 80-Val.fwdarw.Ile,  
84-Ile.fwdarw.Leu, and insertion of Pro and Ser between 84-Ile and  
85-Met. Use of the geranyl- \*\*\*geranyl\*\*\* \*\*\*diphosphate\*\*\*  
\*\*\*synthase\*\*\* \*\*\*mutants\*\*\* for the prepn. of C<15 prenyl  
diphosphates from the reactants such as \*\*\*isopentenyl\*\*\*  
\*\*\*diphosphate\*\*\*, di-Me allyl diphosphate, and \*\*\*geranyl\*\*\*  
\*\*\*diphosphate\*\*\* was also shown.

L7 ANSWER 2 OF 15 CAPLUS COPYRIGHT 1998 ACS

ACCESSION NUMBER: 1998:58895 CAPLUS

DOCUMENT NUMBER: 128:125293

TITLE: \*\*\*Mutants\*\*\* of \*\*\*prenyl\*\*\*  
\*\*\*diphosphate\*\*\* \*\*\*synthase\*\*\* for  
preparation of long-chain prenyl diphosphate

INVENTOR(S): Oto, Toku; Ishida, Chika; Takeuchi, Yoshie;  
Narita, Hiroyuki; Onuma, Shinichi; Nishino,  
Tokuzo

PATENT ASSIGNEE(S): Toyota Motor Corp., Japan

SOURCE: Jpn. Kokai Tokkyo Koho, 17 pp.

CODEN: JKXXAF

NUMBER DATE

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PATENT INFORMATION: JP 10014567 A2 980120 Heisei

APPLICATION INFORMATION: JP 96-191635 960703

DOCUMENT TYPE: Patent

LANGUAGE: Japanese

AB A \*\*\*mutant\*\*\* of \*\*\*prenyl\*\*\* \*\*\*diphosphate\*\*\*  
\*\*\*synthase\*\*\* is prepd. by substituting the residue that is  
5-residue upstream from the Asp-rich domain I DDXX(XX)D (D=Asp; X in  
(XX) may not exist) in the 2nd conserved region. The synthase may  
be \*\*\*farnesyl\*\*\* \*\*\*diphosphate\*\*\* \*\*\*synthase\*\*\* ,  
geranyl- \*\*\*geranyl\*\*\* \*\*\*diphosphate\*\*\* \*\*\*synthase\*\*\* ,  
hexaprenyl \*\*\*diphosphate\*\*\* \*\*\*synthase\*\*\* , heptaprenyl  
\*\*\*diphosphate\*\*\* \*\*\*synthase\*\*\* , octaprenyl  
\*\*\*diphosphate\*\*\* \*\*\*synthase\*\*\* , nonaprenyl  
\*\*\*diphosphate\*\*\* \*\*\*synthase\*\*\* , or undecaprenyl  
\*\*\*diphosphate\*\*\* \*\*\*synthase\*\*\* . The mutant is able to  
catalyze the synthesis of long-chain (C>20) prenyl diphosphate.  
Prepn. of \*\*\*mutants\*\*\* of \*\*\*farnesyl\*\*\*  
\*\*\*diphosphate\*\*\* \*\*\*synthase\*\*\* (FPP synthase) of *Bacillus*  
*stearothermophilus* by substituting 81-Tyr with Asn, Ile, Met, Pro,  
Phe, and Val, resp., was shown.

L7 ANSWER 3 OF 15 BIOSIS COPYRIGHT 1998 BIOSIS

ACCESSION NUMBER: 97:154807 BIOSIS

DOCUMENT NUMBER: 99454010

TITLE: Conversion from archaeal geranylgeranyl  
\*\*\*diphosphate\*\*\* \*\*\*synthase\*\*\* to  
\*\*\*farnesyl\*\*\* \*\*\*diphosphate\*\*\*  
\*\*\*synthase\*\*\* : Two amino acids before the first  
aspartate-rich motif solely determine eukaryotic  
\*\*\*farnesyl\*\*\* \*\*\*diphosphate\*\*\*  
\*\*\*synthase\*\*\* activity.

AUTHOR(S): Ohnuma S-I; Hirooka K; Ohto C; Nishino T

CORPORATE SOURCE: Dep. Biochemistry Engineering, Tohoku Univ., Aoba  
Aramaki, Aoba-ku, Sendai 980-77, Japan

SOURCE: Journal of Biological Chemistry 272 (8). 1997.

5192-5198. ISSN: 0021-9258

LANGUAGE: English

AB \*\*\*Farnesyl\*\*\* \*\*\*diphosphate\*\*\* (FPP) and geranylgeranyl

diphosphate (GGPP) are precursors for a variety of important natural products, such as sterols, carotenoids, and prenyl quinones. Although FPP synthase and GGPP synthase catalyze similar consecutive condensations of \*\*\*isopentenyl\*\*\* \*\*\*diphosphate\*\*\* with allylic diphosphates and have several homologous regions in their amino acid sequences, nothing is known about how these enzymes form the specific products. To locate the region that causes the difference of final products between GGPP synthase and FPP synthase, we constructed six mutated archaeal GGPP synthases whose regions around the first aspartate-rich motif were replaced with the corresponding regions of FPP synthases from human, rat, Arabidopsis thaliana, Saccharomyces cerevisiae, Escherichia coli, Bacillus stearothermophilus, and from some other related mutated enzymes. From the analysis of these mutated enzymes, we revealed that the region around the first aspartate-rich motif is essential for the product specificity of all FPP synthases and that the mechanism of the chain termination in eukaryotic FPP synthases (type I) is different from those of prokaryotic FPP synthases (type II). In FPP synthases of type I, two amino acids situated at the fourth and the fifth positions before the motif solely determine their product chain length, while the product specificity of the type II enzymes is determined by one aromatic amino acid at the fifth position before the motif, two amino acids inserted in the motif, and other modifications. These data indicate that FPP synthases have evolved from the progenitor corresponding to the archaeal GGPP synthase in two ways.

L7 ANSWER 4 OF 15 CAPLUS COPYRIGHT 1998 ACS

ACCESSION NUMBER: 1997:170512 CAPLUS

DOCUMENT NUMBER: 126:183141

TITLE: Crystal structure of recombinant avian wild type and \*\*\*mutant\*\*\* \*\*\*farnesyl\*\*\* \*\*\*diphosphate\*\*\* \*\*\*synthase\*\*\* and the binding modes of substrates and inhibitor compounds (prenyltransferases)

AUTHOR(S): Tarshis, Larry

CORPORATE SOURCE: Yeshiva Univ., New York, NY, USA

SOURCE: (1996) 486 pp. Avail.: Univ. Microfilms Int., Order No. DA9705286

From: Diss. Abstr. Int., B 1997, 57(9), 5632

DOCUMENT TYPE: Dissertation

LANGUAGE: English

AB Unavailable

L7 ANSWER 5 OF 15 CAPLUS COPYRIGHT 1998 ACS

ACCESSION NUMBER: 1996:635257 CAPLUS

DOCUMENT NUMBER: 125:269265

TITLE: \*\*\*Mutant\*\*\* \*\*\*farnesyl\*\*\* \*\*\*diphosphate\*\*\* \*\*\*synthase\*\*\* genes of Bacillus stearothermophilus, their preparation, and use in synthesizing geranylgeranyl diphosphate

INVENTOR(S): Ayumi, Koike; Tokuzo, Nishino; Shusei, Obata; Shinichi, Ohnuma; Takeshi, Nakazawa; Kyoze,

Ogura; Tanetoshi, Koyama  
PATENT ASSIGNEE(S): Toyota Jidosha Kabushiki Kaisha, Japan  
SOURCE: Eur. Pat. Appl., 50 pp.  
CODEN: EPXXDW

NUMBER	DATE
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PATENT INFORMATION:	EP 733709 A2 960925
DESIGNATED STATES:	R: BE, CH, DE, FR, GB, IT, LI, SE
APPLICATION INFORMATION:	EP 95-115423 950929
PRIORITY APPLN. INFO.:	JP 95-25253 950214
DOCUMENT TYPE:	Patent
LANGUAGE:	English
AB The genes encoding 4 ***farnesyl*** ***diphosphate*** ***synthase*** ***mutants*** were prep'd. by treating the wild type gene of Bacillus stearothermophilus with Na nitrite. These mutants have .gtoreq.1 mutations at position 34, 59, 81, 157, 182, 239, or 275 corresponding the wild type ***farnesyl*** ***diphosphate*** ***synthase*** . The mutated enzymes were produced in transgenic Escherichia coli. The mutants produced an amt. of geranylgeranyl diphosphate more than that of ***farnesyl*** ***diphosphate*** .	

L7 ANSWER 6 OF 15 MEDLINE DUPLICATE 1  
ACCESSION NUMBER: 96324966 MEDLINE  
DOCUMENT NUMBER: 96324966  
TITLE: Conversion of product specificity of archaeobacterial  
geranylgeranyl- \*\*\*diphosphate\*\*\* \*\*\*synthase\*\*\*  
. Identification of essential amino acid residues for  
chain length determination of prenyltransferase  
reaction.  
AUTHOR: Ohnuma S; Hirooka K; Hemmi H; Ishida C; Ohto C;  
Nishino T  
CORPORATE SOURCE: Department of Biochemistry and Engineering, Tohoku  
University, Aoba Aramaki, Aoba-ku, Sendai 980-77,  
Japan.  
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1996 Aug 2) 271  
(31) 18831-7.  
Journal code: HIV. ISSN: 0021-9258.  
PUB. COUNTRY: United States  
Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals; Cancer Journals  
ENTRY MONTH: 199611  
AB Prenyltransferases catalyze the consecutive condensation of  
\*\*\*isopentenyl\*\*\* \*\*\*diphosphate\*\*\* with allylic diphosphates  
to produce prenyl diphosphates whose chain lengths are absolutely  
determined by each enzyme. To investigate the mechanism of the  
consecutive reaction and the determination of the ultimate chain  
length, a random mutational approach was planned. A geranylgeranyl-  
\*\*\*diphosphate\*\*\* \*\*\*synthase\*\*\* gene from Sulfolobus  
acidocaldarius was randomly mutagenized by NaNO2 treatment to  
construct a library of mutated geranylgeranyl- \*\*\*diphosphate\*\*\*  
\*\*\*synthase\*\*\* genes on a yeast expression vector. The library was



screened for suppression of a pet phenotype of yeast C296-LH3, which is deficient in hexaprenyl- \*\*\*diphosphate\*\*\* \*\*\*synthase\*\*\*. Five \*\*\*mutants\*\*\* that could grow on a YEPG plate, which contained only glycerol as an energy source instead of glucose, were selected from approximately 1,400 mutants. All selected mutated enzymes catalyzed the formation of polyprenyl diphosphates with prenyl chains longer than geranylgeranyl diphosphate. Especially mutants 1, 3, and 5 showed the strongest elongation activity to produce large amounts of geranylfarnesyl diphosphate with a concomitant amount of hexaprenyl diphosphate. Sequence analysis revealed that each mutant contained a few amino acid substitutions and that the mutation of Phe-77, which is located on the fifth amino acid upstream from the first aspartate-rich consensus motif, is the most effective for elongating the ultimate product. Amino acid alignment of known prenyltransferases around this position and our previous observations on \*\*\*farnesyl\*\*\* - \*\*\*diphosphate\*\*\* \*\*\*synthase\*\*\* (Ohnuma, S.-i., Nakazawa, T., Hemmi, H., Hallberg, A.-M., Koyama, T., Ogura, K., and Nishino, T. (1996) J. Biol. Chem. 271, 10087-10095) clearly indicate that the amino acid at the position of all prenyltransferases must regulate the chain elongation.

L7 ANSWER 7 OF 15 CAPLUS COPYRIGHT 1998 ACS DUPLICATE 2

ACCESSION NUMBER: 1996:388315 CAPLUS

DOCUMENT NUMBER: 125:52249

TITLE: Identification of significant residues in the substrate binding site of Bacillus stearothermophilus \*\*\*farnesyl\*\*\* \*\*\*diphosphate\*\*\* \*\*\*synthase\*\*\*

AUTHOR(S): Koyama, Tanetoshi; Tajima, Masaya; Sano, Hiroaki; Doi, Takashi; Koike-Takeshita, Ayumi; Obata, Shusei; Nishino, Tokuzo; Ogura, Kyoza

CORPORATE SOURCE: Faculty of Engineering, Tohoku University, Sendai, 980-77, Japan

SOURCE: Biochemistry (1996), 35(29), 9533-9538  
CODEN: BICHAW; ISSN: 0006-2960

DOCUMENT TYPE: Journal

LANGUAGE: English

OTHER SOURCE(S): CJACS-IMAGE; CJACS

AB \*\*\*Farnesyl\*\*\* \*\*\*diphosphate\*\*\* \*\*\*synthase\*\*\* (I) from a wide range of organisms was previously shown to possess 7 highly conserved regions (I-VII) in the amino acid sequence. Site-directed mutants of I from B. stearothermophilus were made to evaluate the roles of the conserved Asp residues in region VI and Lys residues in regions I, V, and VI. Asp-224 was changed to Ala or Glu (mutants D224A and D224E, resp.); Asp-225 and Asp-228 were changed to Ile and Ala (D225I and D228A, resp.); Lys-238 was changed to either Ala or Arg (K238A or K238R, resp.). Lys-47 and Lys-183 were changed to Ile and Ala (K47I and K183A, resp.). Kinetic analyses of the wild-type and mutant enzymes indicated that mutagenesis of Asp-224 and Asp-225 resulted in a decrease in kcat values of approx. 104- to 105-fold compared to wild-type I. On the other hand, D228A showed a kcat .apprx. 1/10 of that of wild-type I, and the Km for \*\*\*isopentenyl\*\*\* \*\*\*diphosphate\*\*\* increased .apprx. 10-fold.

Both K47I and K183A mutants exhibited Km values for  
\*\*\*isopentenyl\*\*\* \*\*\*diphosphate\*\*\* 20-fold larger and kcat  
values 70-fold smaller than did wild-type I. These results suggest  
that the 2 conserved Lys residues in regions I and V contribute to  
the binding of \*\*\*isopentenyl\*\*\* \*\*\*diphosphate\*\*\* and that  
the 1st and the 2nd Asp residues in region VI are involved in  
catalytic function. Asp-228 is also important for the binding of  
\*\*\*isopentenyl\*\*\* \*\*\*diphosphate\*\*\* rather than for the  
catalytic reaction.

L7 ANSWER 8 OF 15 CAPLUS COPYRIGHT 1998 ACS

ACCESSION NUMBER: 1996:703698 CAPLUS

DOCUMENT NUMBER: 126:16162

TITLE: Substrate specificities of wild and mutant FPP  
synthases from Bacillus stearothermophilus

AUTHOR(S): Maki, Y.; Shimizu, K.; Arai, H.; Ono, H.;  
Koyama, T.; Ogura, K.

CORPORATE SOURCE: Department Chemistry, Yamagata University,  
Yamagata, 990, Japan

SOURCE: Tennen Yuki Kagobutsu Toronkai Koen Yoshishu  
(1996), 38th, 295-300

CODEN: TYKYDS

PUBLISHER: Nippon Kagakkai

DOCUMENT TYPE: Journal

LANGUAGE: Japanese

AB Substrate specificity of FPP synthases (I) of B. stearothermophilus  
is studied with several synthetic substrates. The substrate  
specificity is very similar to that of chicken liver and pig liver.  
The cysteine residues in position-73 and -289 are not assocd. with  
the enzymic activity. However, the Gln residue in position-221 is  
involved in the binding of allylic substrates and catalysis of I.

L7 ANSWER 9 OF 15 CAPLUS COPYRIGHT 1998 ACS DUPLICATE 3

ACCESSION NUMBER: 1994:403883 CAPLUS

DOCUMENT NUMBER: 121:3883

TITLE: Yeast \*\*\*farnesyl\*\*\* - \*\*\*diphosphate\*\*\*  
\*\*\*synthase\*\*\* : Site-directed mutagenesis of  
residues in highly conserved prenyltransferase  
domains I and II

AUTHOR(S): Song, Linsheng; Poulter, C. Dale

CORPORATE SOURCE: Dep. Chem., Univ. Utah, Salt Lake City, UT,  
84112, USA

SOURCE: Proc. Natl. Acad. Sci. U. S. A. (1994), 91(8),  
3044-8

CODEN: PNASA6; ISSN: 0027-8424

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Prenyltransferases that catalyze the fundamental chain elongation  
reactin in the isoprenoid biosynthetic pathway contain several  
highly conserved amino acids, including two aspartate-rich regions  
thought to be involved in substrate binding and catalysis. The  
authors report a study of site-directed \*\*\*mutants\*\*\* for yeast  
\*\*\*farnesyl\*\*\* - \*\*\*diphosphate\*\*\* \*\*\*synthase\*\*\* (FPPSase;  
\*\*\*geranyl\*\*\* - \*\*\*diphosphate\*\*\* : \*\*\*isopentenyl\*\*\* -

\*\*\*diphosphate\*\*\*, EC 2.5.1.10), a prenyltransferase that catalyzes the sequential 1'-4 coupling of \*\*\*isopentenyl\*\*\* \*\*\*diphosphate\*\*\* (IPP) with \*\*\*dimethylallyl\*\*\* \*\*\*diphosphate\*\*\* and \*\*\*geranyl\*\*\* \*\*\*diphosphate\*\*\*. A recombinant form of FPPSase extended by a C-terminal -Glu-Glu-Phe .alpha.-tubulin epitope (EEF in single-letter amino acid code) was engineered to facilitate rapid purifn. of the enzyme by immunoaffinity chromatog. and to remove traces of contaminating activity from wild-type FPPSase in the Escherichia coli host. Ten site-directed mutants were constructed in FPPSase::EEF. The six aspartates in domain I (at positions 100, 101, and 104) and domain II (at positions 240, 241, and 244) were changed to alanine (mutants designated D100A, D101A, D104A, D240A, D241A, and D244A); three arginine residues were changed, Arg-109 and Arg-110 to glutamine and Arg-350 to alanine (mutants designated R109Q, R110Q, and R350A); and Lys-254 was converted to alanine (mutant designated K254A). Mutations of the aspartate residues and nearby arginine residues in domain I and Asp-240 and Asp-241 in domain II drastically lowered the catalytic activity of FPPSase::EEF. The D244A and K254A mutants were substantially less active, while kcat and the Michaelis consts. for the R350A mutant were similar to those of FPPSase::EEF. Addn. of an -EEF epitope to the C terminus of wild-type FPPSase resulted in a 14-fold increase of KmIPP and a 12-fold decrease of kcat, suggesting that the conserved hydrophilic C terminus of the enzyme may have a role in substrate binding and catalysis.

L7 ANSWER 10 OF 15 CAPLUS COPYRIGHT 1998 ACS

ACCESSION NUMBER: 1994:403855 CAPLUS

DOCUMENT NUMBER: 121:3855

TITLE: Site-directed mutagenesis of \*\*\*farnesyl\*\*\* \*\*\*diphosphate\*\*\* \*\*\*synthase\*\*\*; effect of substitution on the three carboxyl-terminal amino acids

AUTHOR(S): Koyama, Tanetoshi; Saito, Kazuhiro; Ogura, Kyozo; Obata, Shusei; Takeshita, Ayumi

CORPORATE SOURCE: Inst. Chem. React. Sci., Tohoku Univ., Sendai, 980, Japan

SOURCE: Can. J. Chem. (1994), 72(1), 75-9

CODEN: CJCHAG; ISSN: 0008-4042

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Site-directed mutation was introduced into the gene for the \*\*\*farnesyl\*\*\* \*\*\*diphosphate\*\*\* \*\*\*synthase\*\*\* of Bacillus stearothermophilus. To investigate the significance of the three C-terminal amino acids, where arginine is completely conserved throughout the \*\*\*farnesyl\*\*\* \*\*\*diphosphate\*\*\* \*\*\*synthases\*\*\* of prokaryotes and eukaryotes, three kinds of mutant enzymes, R295V, D296G, and H297L, which have replacements of arginine-295 with valine, aspartate-296 with glycine, and histidine-297 with leucine, resp., were overproduced and purified to homogeneity. All of the three mutant enzymes showed similar catalytic activities to that of the wild-type enzyme, indicating that the basic amino acids including the conserved arginine in the C-terminal region are not essential for catalytic function. They

were also similar to the wild-type enzyme with respect to pH optima, thermostability, reaction product, and kinetic parameters for allylic substrates. However, their  $K_m$  values for \*\*\*isopentenyl\*\*\* \*\*\*diphosphate\*\*\* are approx. twice that of the wild type.

L7 ANSWER 11 OF 15 CAPLUS COPYRIGHT 1998 ACS

ACCESSION NUMBER: 1993:644327 CAPLUS

DOCUMENT NUMBER: 119:244327

TITLE: Effect of site-directed mutagenesis of conserved aspartate and arginine residues upon \*\*\*farnesyl\*\*\* \*\*\*diphosphate\*\*\* \*\*\*synthase\*\*\* activity

AUTHOR(S): Joly, Alison; Edwards, Peter A.

CORPORATE SOURCE: Dep. Biol. Chem., Univ. California, Los Angeles, CA, 90024, USA

SOURCE: J. Biol. Chem. (1993), 268(36), 26983-9  
CODEN: JBCHA3; ISSN: 0021-9258

DOCUMENT TYPE: Journal

LANGUAGE: English

AB All polyprenyl synthases catalyze the condensation of the allylic substrate, \*\*\*isopentenyl\*\*\* \*\*\*diphosphate\*\*\*, with a specific homoallylic diphosphate substrate. Polyprenyl synthases from *Homo sapiens*, *R. rattus*, *Escherichia coli*, *Saccharomyces cerevisiae*, *N. crassa*, and *E. herbicola* contain two conserved "aspartate-rich domains" (Ashby, M. N., and Edwards, P. A. (1992) J. Biol. Chem. 267, 4128-4136). In order to det. the importance of these domains in catalysis, the conserved aspartates or arginines in domains I and II of rat \*\*\*farnesyl\*\*\* \*\*\*diphosphate\*\*\* \*\*\*synthase\*\*\* were individually mutated to glutamate or lysine, resp. The putative "active site" arginine (Brems, D. N., Breunger, E., and Rilling, H. C. (1981) Biochem. 20, 3711-3718) was mutated to lysine. Each mutant enzyme was overexpressed in *E. coli* and purified to apparent homogeneity. Detailed kinetic analyses of the wild type and mutant enzymes indicated that mutagenesis of Asp104, Asp107, Arg112, Arg113, and Asp243 resulted in a decreased  $V_{max}$  of approx. 1000-fold compared to wild type. However, no significant change in the  $K_m$  values for either the \*\*\*isopentenyl\*\*\* \*\*\*diphosphate\*\*\* or \*\*\*geranyl\*\*\* \*\*\*diphosphate\*\*\* substrate were obsd. The results strongly suggest that these amino acids, and to a lesser extent Asp244, are involved in either the condensation of \*\*\*isopentenyl\*\*\* \*\*\*diphosphate\*\*\* and \*\*\*geranyl\*\*\* \*\*\*diphosphate\*\*\* to form \*\*\*farnesyl\*\*\* \*\*\*diphosphate\*\*\* and/or the release of the \*\*\*farnesyl\*\*\* \*\*\*diphosphate\*\*\* product from \*\*\*farnesyl\*\*\* \*\*\*diphosphate\*\*\* \*\*\*synthase\*\*\*. The conservation of these amino acid residues in different enzymes from several species suggests that these domains play a similar role in other polyprenyl synthases.

L7 ANSWER 12 OF 15 CAPLUS COPYRIGHT 1998 ACS

ACCESSION NUMBER: 1993:423605 CAPLUS

DOCUMENT NUMBER: 119:23605

TITLE: Characterization of a lysine-to-glutamic acid

mutation in a conservative sequence of

\*\*\*farnesyl\*\*\* \*\*\*diphosphate\*\*\*

\*\*\*synthase\*\*\* from *Saccharomyces cerevisiae*

AUTHOR(S): Blanchard, Laurence; Karst, Francis

CORPORATE SOURCE: Inst. Biol. Mol. Ing. Genet., Univ. Poitiers,  
Poitiers, 86022, Fr.

SOURCE: Gene (1993), 125(2), 185-9

CODEN: GENED6; ISSN: 0378-1119

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The mutant gene *erg20-2* was isolated from a yeast strain defective  
in \*\*\*farnesyl\*\*\* \*\*\*diphosphate\*\*\* \*\*\*synthase\*\*\* (I).

This strain had the unusual property of excreting prenyl alcs., such  
as geraniol. The nucleotide (nt) sequence, compared with that of  
the wild-type gene, showed a single nt change, resulting in a  
Lys-197 → Phe. Glu substitution in I which was directly involved  
in terpenic alc. formation. In addn., disruption of *ERG20* revealed  
that in yeast no other prenyltransferase is able to synthesize the  
\*\*\*farnesyl\*\*\* \*\*\*diphosphate\*\*\* mols. required for essential  
nonsterol metabolites.

L7 ANSWER 13 OF 15 CAPLUS COPYRIGHT 1998 ACS

ACCESSION NUMBER: 1992:587237 CAPLUS

DOCUMENT NUMBER: 117:187237

TITLE: Effects of site-directed mutagenesis of the  
highly conserved aspartate residues in domain II  
of \*\*\*farnesyl\*\*\* - \*\*\*diphosphate\*\*\*  
\*\*\*synthase\*\*\* activity

AUTHOR(S): Marrero, Pedro F.; Poulter, C. Dale; Edwards,  
Peter A.

CORPORATE SOURCE: Dep. Biol. Chem., UCLA, Los Angeles, CA, 90024,  
USA

SOURCE: J. Biol. Chem. (1992), 267(30), 21873-8

CODEN: JBCHA3; ISSN: 0021-9258

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Comparison of the \*\*\*farnesyl\*\*\* \*\*\*diphosphate\*\*\*  
\*\*\*synthase\*\*\* (I) amino acid sequences from 4 species with amino  
acid sequences from the related enzymes, hexaprenyl

\*\*\*diphosphate\*\*\* \*\*\*synthase\*\*\* and geranylgeranyl  
diphosphate (GPP) synthase showed the presence of 2 aspartate-rich  
highly conserved domains. The aspartate motif [(I, L, or V)XDDXXD]  
of the 2nd of those domains exhibited homol. with at least 9  
prenyl-transfer enzymes that utilize an allylic prenyl diphosphate  
as 1 substrate. In order to investigate the role of this 2nd  
aspartate-rich domain in rat I, the 1st or 3rd aspartate was mutated  
to glutamate, the wild-type and mutant enzymes were expressed in  
*Escherichia coli*, and the enzymes were purified to apparent  
homogeneity using a single chromatog. step. Approx. 12 mg of  
homogeneous protein was isolated from 120 mg of crude bacterial ext.  
The kinetic parameters of purified wild-type recombinant I contg.  
the DDYLD motif were as follows:  $V_{max} = 0.84 \text{ } \mu\text{mol/min/mg}$ ; GPP  $K_m$   
 $= 1.0 \text{ } \mu\text{M}$ ; \*\*\*isopentenyl\*\*\* \*\*\*diphosphate\*\*\* (IPP)  $K_m$  =  
 $2.7 \text{ } \mu\text{M}$ . Substitution of glutamate for the 1st aspartate (EDYLD)

decreased the Vmax by >90-fold. The Km for IPP increased, whereas the Km for GPP remained the same in this D243E mutant. Substitution of glutamate for the 3rd aspartate (DDYLE) did not result in altered enzyme kinetics in the D247E mutant. These results suggest that the 1st aspartate (Asp-243) in the 2nd domain is involved in the catalysis by I.

L7 ANSWER 14 OF 15 CAPLUS COPYRIGHT 1998 ACS

ACCESSION NUMBER: 1990:96998 CAPLUS

DOCUMENT NUMBER: 112:96998

TITLE: Process for obtaining terpenic aromas by a microbiological process

INVENTOR(S): Karst, Francis; Vladescu, Barbu Dinu Vladimir

PATENT ASSIGNEE(S): Pernod-Ricard S. A., Fr.

SOURCE: Eur. Pat. Appl., 8 pp.

CODEN: EPXXDW

NUMBER	DATE
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PATENT INFORMATION:	EP 313465 A1	890426
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DESIGNATED STATES:	R: AT, BE, CH, DE, ES, FR, GB, GR, IT, LI, LU, NL, SE
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APPLICATION INFORMATION:	EP 88-402647	881020
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PRIORITY APPLN. INFO.:	FR 87-14609	871022
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DOCUMENT TYPE: Patent

LANGUAGE: French

AB *Saccharomyces cerevisiae* mutants which are blocked in the ergosterol synthetic pathway and which consequently secrete terpenoid aromas, e.g. geraniol, farnesol, linalool, are produced. Such mutants are further mutagenized to produce double mutants which are addnl. defective in alc. dehydrogenase I (ADH-I) or ADH-IIe. The former may be used to pepd. flavored fruit juice, milk, or cereal must. The latter may be used to prep. sparkling wine. Thus, a mutant deficient in squalene synthetase activity (requires ergosterol for growth) and ADH-I was prepd. This mutant, erg 9, was used to produce fruit juices with various flavors/aromas due to its prodn. of farnesol.

L7 ANSWER 15 OF 15 CAPLUS COPYRIGHT 1998 ACS DUPLICATE 4

ACCESSION NUMBER: 1990:4293 CAPLUS

DOCUMENT NUMBER: 112:4293

TITLE: Isolation and characterization of an *Escherichia coli* \*\*\*mutant\*\*\* having temperature-sensitive \*\*\*farnesyl\*\*\* \*\*\*diphosphate\*\*\* \*\*\*synthase\*\*\*

AUTHOR(S): Fujisaki, Shingo; Nishino, Tokuzo; Katsuki, Hirohiko; Hara, Hiroshi; Nishimura, Yukinobu; Hirota, Yukinori

CORPORATE SOURCE: Fac. Gen. Educ., Gifu Univ., Gifu, 501-11, Japan

SOURCE: J. Bacteriol. (1989), 171(10), 5654-8

CODEN: JOBAAY; ISSN: 0021-9193

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The screening of a collection of highly mutagenized strains of *E.*

coli for defects in isoprenoid synthesis led to the isolation of a  
\*\*\*mutant\*\*\* that had temp.-sensitive \*\*\*farnesyl\*\*\*  
\*\*\*diphosphate\*\*\* \*\*\*synthase\*\*\*. The defective gene, named  
ispA, was mapped at about min 10 on the E. coli chromosome, and the  
gene order was shown to be tsx-ispA-lon. The mutant ispA gene was  
transferred to the E. coli strain with a defined genetic background  
by P1 transduction for investigation of its function. The in vitro  
activity of \*\*\*farnesyl\*\*\* \*\*\*diphosphate\*\*\*  
\*\*\*synthase\*\*\* of the \*\*\*mutant\*\*\* was 21% of that of the  
wild-type strain at 30.degree. and 5% of that at 40.degree.. At  
42.degree. the ubiquinone level was lower (66% of normal) in the  
mutant than in the wild-type strain, whereas at 30.degree. the level  
in the mutant was almost equal to that in the wild-type strain. The  
polyprenyl phosphate level was slightly higher in the mutant than in  
the wild-type strain at 30.degree. and almost the same in both  
strains at 42.degree.. The mutant had no obvious phenotype